



1 Figs. 6A-6E are graphs showing the results of ELISA analyses for cytokines and  
2 chemokines released from normal human microglia and HMO6 immortalized human  
3 microglia cells; and

4 Fig. 7 is a photograph shows the cytogenetic analysis of HMO6 immortalized human  
5 microglia cells as the normal karyotype of human cells.

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7 DETAILED DESCRIPTION OF THE INVENTION

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9 The present invention is the establishment and characterization of several continuous  
10 cell lines of immortalized human microglia, labeled as HMO6, generated by transfection of  
11 embryonic (fetal) human microglia (HM) with a retroviral vector containing cDNA for the v-  
12 myc oncogene. The invention provides a phenotypic characterization of these immortalized  
13 human microglia; and discloses the expression of cytokines and chemokines following  
14 exposure to  $\beta$  amyloid peptides using HM and HMO6 cells. For a clearer understanding and  
15 better appreciation of the subject matter as a whole which comprises the present invention,  
16 the detailed description will be presented as separate sections.

17  
18 I. A Preferred Method For Producing Immortalized Human Microglia Cells And  
19 Continuous Cell Lines  
20

21 'Human microglial cell line, as used herein, means a human-derived cell line with  
22 microglial characteristics, including at least the specific antigens CD68 and CD11b. Also, as  
23 used herein, "non-fetal" refers to the fact that the progeny cells are expanded from



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Table E1: Sequences of PCR Primers

	<u>Gene</u>	<u>Sequence</u>	<u>Product Size</u> (bp)
1			
2			
3			
4			
5			
6	CD68 sense	AGATTCGAGTCATGTACACAACCCA [SEQ ID NO:1]	279
7	CD68 antisense	GGTGCTTGGAGATCTCGAAG [SEQ ID NO:2]	
8			
9	P <sub>2Y1</sub> R sense	TGTGGTGTACCCCCTCAAGTCCC [SEQ ID NO:3]	260
10	P <sub>2Y1</sub> R antisense	ATCCGTAACAGCCCAGAATCAGCA [SEQ ID NO:4]	
11			
12	P <sub>2Y2</sub> R sense	CCAGGCCCCCGTGCTCTACTTTG [SEQ ID NO:5]	367
13	P <sub>2Y2</sub> R antisense	CATGTTGATGGCGTTGAGGGTGTG [SEQ ID NO:6]	
14			
15	CXCR4 sense	TTCTACCCCAATGACTTGTG [SEQ ID NO:7]	206
16	CXCR4 antisense	ATGTAGTAAGGCAGCCAACA [SEQ ID NO:8]	
17			
18	MIP-1 $\alpha$ sense	ACCATGGCTCTCTGCAACCA [SEQ ID NO:9]	393
19	MIP-1 $\alpha$ antisense	TTAAGAAGAGTCCCACAGTG [SEQ ID NO:10]	
20			
21	MIP-1 $\beta$ sense	CCTGCTGCTTTTCTTACACC [SEQ ID NO:11]	336
22	MIP-1 $\beta$ antisense	CACCTAATAACAATAACACGGC [SEQ ID NO:12]	
23			
24	MCP-1 sense	ATAGCAGCCACCTTCATTCC [SEQ ID NO:13]	466
25	MCP-1 antisense	TTCCCCAAGTCTCTGTATCT [SEQ ID NO:14]	
26			
27	IL-1 $\beta$ sense	AAAAGCTTGGTGATGTCTGG [SEQ ID NO:15]	179
28	IL-1 $\beta$ antisense	TTTCAACACGCAGGACAGG [SEQ ID NO:16]	
29			
30	IL-2 sense	ATGGTTGCTGTCTCATCAGC [SEQ ID NO:17]	301
31	IL-2 antisense	CTGGAGCATTTACTGCTGGA [SEQ ID NO:18]	
32			
33	IL-3 sense	ATGAGCCGCTGCCCCGTCCTG [SEQ ID NO:19]	459
34	IL-3 antisense	AAGATCGCGAGGCTCAAAGTCGTCTGTTG [SEQ ID NO:20]	
35			
36	IL-4 sense	GACACAAGTGCAATATCACC [SEQ ID NO:21]	337
37	IL-4 antisense	AAGTTTTCCAACGTA CTCTG [SEQ ID NO:22]	
38			
39	IL-5 sense	GAGGATGCTTCTGCATTTGAGTTTG [SEQ ID NO:23]	295
40	IL-5 antisense	GTCAATGTATTTCTTTATTAAGGACAAG [SEQ ID NO:24]	
41			
42	IL-6 sense	GTGTGAAAGCAGCAAAGAGGC [SEQ ID NO:25]	159
43	IL-6 antisense	CTGGAGGTACTCTAGGTATAC [SEQ ID NO:26]	
44			

Table E1: Sequences of PCR Primers (continued)

<u>Gene</u>	<u>Sequence</u>	<u>Product Size</u> (bp)
IL-7 sense	TGTTGAACTGCACTGGCCAG [SEQ ID NO:27]	484
IL-7 antisense	GCAACTGATACCTTACATGG [SEQ ID NO:28]	
IL-8 sense	ATGACTTCCAAGCTGGCCGTG [SEQ ID NO:29]	301
IL-8 antisense	TATGAATTCTCAGCCCTCTTCAAAA [SEQ ID NO:30]	
IL-9 sense	ATGCTTCTGGCCATGGTCCT [SEQ ID NO:31]	375
IL-9 antisense	TATCTTGCCTCTCATCCCTC [SEQ ID NO:32]	
IL-10 sense	AGATCTCCGAGATGCCTTCAGCAGA [SEQ ID NO:33]	194
IL-10 antisense	CCTTGATGTCTGGGTCTTGGTTCTC [SEQ ID NO:34]	
IL-11 sense	ACTGCTGCTGCTGAAGACTCGGCTGTGA [SEQ ID NO:35]	295
IL-11 antisense	ATGGGGAAGAGCCAGGGCAGAAGTCTGT [SEQ ID NO:36]	
IL-12 sense	TCACAAAGGAGGCGAGGTTCTAAGC [SEQ ID NO:37]	213
IL-12 antisense	CCTCTGCTGCTTTTGACACTGAATG [SEQ ID NO:38]	
IL-13 sense	ACCCAGAACCAGAAGGCTCCG [SEQ ID NO:39]	198
IL-13 antisense	TCAGTTGAACCGTCCCTGGCG [SEQ ID NO:40]	
IL-15 sense	AAACCCCTGCCATAGCCAACTCTT [SEQ ID NO:41]	202
IL-15 antisense	CTTCTGTTTTAGGGAGCCCTGCACT [SEQ ID NO:42]	
TNF- $\alpha$ sense	CAAAGTAGACCTGCCCAGAC [SEQ ID NO:43]	490
TNF- $\alpha$ antisense	GACCTCTCTCTAATCAGCCC [SEQ ID NO:44]	
NF-M sense	TGGGAAATGGCTCGTCATTT [SEQ ID NO:45]	333
NF-M antisense	CTTCATGGAAGCGGCCAATT [SEQ ID NO:46]	
MBP sense	ACACGGGCATCCTTGACTCCATCGG [SEQ ID NO:47]	510
MBP antisense	TCCGGAACCAGGTGGGTTTTTCAGCG [SEQ ID NO:48]	
GFAP sense	GCAGAGATGATGGAGCTCAATGACC [SEQ ID NO:49]	266
GFAP antisense	GTTTCATCCTGGAGCTTCTGCCTCA [SEQ ID NO:50]	
B7-2 sense	CTCTTTGTGATGGCCTTCCTG [SEQ ID NO:51]	464
B7-2 antisense	CTTAGGTCTGGGTAACCGTG [SEQ ID NO:52]	

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Table E1: Sequences of PCR Primers (continued)

<u>Gene</u>	<u>Sequence</u>	<u>Product Size</u>
G3PDH sense	CCATGTTCGTCATGGGTGTGAACCA [SEQ ID NO:53]	251
G3PDH antisense	GCCAGTAGAGGCAGGGATGATGTTC [SEQ ID NO:54]	

bp = base pairs.

1       **Gene expression of cytokines and chemokines following AB treatment**

2       Gene expression of cytokines and chemokines in HM or HMO6.A1 cells was  
3       examined following a 6 hr treatment with or without 20  $\mu$ M of A $\beta$ <sub>25-35</sub> (NH<sub>2</sub>-  
4       GSNKGAIIGLM-COOH) [SED ID NO:55]. LPS at 100 ng/ml was used in microglial cultures  
5       since LPS is a potent activator of microglia [Gebicke-Jiacter *J. Neurosci.* 9: 187-194 (1989);  
6       Suzumuraetal., *Brain Res.* 545: 301-306 (1991)].

7  
8       **ELISA analysis**

9       Production of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8 or MIP-1 $\alpha$  in normal human microglial cells  
10      or HMO6.A1 cells was determined in spent culture supernatants using ELISA kits specific  
11      for human TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8 or MIP-1 $\alpha$  (R&D Systems, capable of detecting TNF- $\alpha$   
12      at 4.4 pg/ml, IL-1 $\beta$  at 1 pg/ml, IL-6 at 0.70 pg/ml, IL-8 at 10 pg/ml and MIP-1 $\alpha$  at 10 pg/ml).  
13      At the end of each experiment, culture supernatants were collected, centrifuged, and stored at  
14      -70CC.

15

16                               Experimental Series I: Isolation of human microglia cell lines

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18      Microglial-enriched populations were isolated from primary cultures of embryonic  
19      human telencephalon cells by virtue of differences in dish-adherent properties. The major  
20      differences are shown by Figs. 2A-2D respectively.

21      Fig. 2 as a whole shows the morphological appearance, and antigenic and functional  
22      tests of HM and HMO6.A1 cells. Fig. 2A is a phase contrast microscopy of HM; and Fig. 2B